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Review

Elevated CO_2 and plant structure: a review

SETH G. PRITCHARD,* HUGO H. ROGERS,* STEPHEN A. PRIOR* and CURT M. PETERSON[1]

**USDA-ARS National Soil Dynamics Laboratory, 411 South Donahue Drive, Auburn, AL 36832, USA, [1]Department of Biological Sciences, University of Northern Colorado, Greeley, CO 80639, USA.*

Abstract

Consequences of increasing atmospheric CO_2 concentration on plant structure, an important determinant of physiological and competitive success, have not received sufficient attention in the literature. Understanding how increasing carbon input will influence plant developmental processes, and resultant form, will help bridge the gap between physiological response and ecosystem level phenomena. Growth in elevated CO_2 alters plant structure through its effects on both primary and secondary meristems of shoots and roots. Although not well established, a review of the literature suggests that cell division, cell expansion, and cell patterning may be affected, driven mainly by increased substrate (sucrose) availability and perhaps also by differential expression of genes involved in cell cycling (e.g. cyclins) or cell expansion (e.g. xyloglucan endotransglycosylase). Few studies, however, have attempted to elucidate the mechanistic basis for increased growth at the cellular level.

Regardless of specific mechanisms involved, plant leaf size and anatomy are often altered by growth in elevated CO_2, but the magnitude of these changes, which often decreases as leaves mature, hinges upon plant genetic plasticity, nutrient availability, temperature, and phenology. Increased leaf growth results more often from increased cell expansion rather than increased division. Leaves of crop species exhibit greater increases in leaf thickness than do leaves of wild species. Increased mesophyll and vascular tissue cross-sectional areas, important determinates of photosynthetic rates and assimilate transport capacity, are often reported. Few studies, however, have quantified characteristics more reflective of leaf function such as spatial relationships among chlorenchyma cells (size, orientation, and surface area), intercellular spaces, and conductive tissue. Greater leaf size and/or more leaves per plant are often noted; plants grown in elevated CO_2 exhibited increased leaf area per plant in 66% of studies, compared to 28% of observations reporting no change, and 6% reported a decrease in whole plant leaf area. This resulted in an average net increase in leaf area per plant of 24%. Crop species showed the greatest average increase in whole plant leaf area (+37%) compared to tree species (+14%) and wild, nonwoody species (+15%). Conversely, tree species and wild, nontrees showed the greatest reduction in specific leaf area (−14% and −20%) compared to crop plants (−6%).

Alterations in developmental processes at the shoot apex and within the vascular cambium contributed to increased plant height, altered branching characteristics, and increased stem diameters. The ratio of internode length to node number often increased, but the length and sometimes the number of branches per node was greater, suggesting reduced apical dominance. Data concerning effects of elevated CO_2 on stem/branch anatomy, vital for understanding potential shifts in functional relationships of leaves with stems, roots with stems, and leaves with roots, are too few to

Correspondence: Dr Seth G. Pritchard, tel +1/334-844-4741 ext. 142, fax +1/334-887-8597, e-mail pritcsg@mail.auburn.edu

make generalizations. Growth in elevated CO_2 typically leads to increased root length, diameter, and altered branching patterns. Altered branching characteristics in both shoots and roots may impact competitive relationships above and below the ground.

Understanding how increased carbon assimilation affects growth processes (cell division, cell expansion, and cell patterning) will facilitate a better understanding of how plant form will change as atmospheric CO_2 increases. Knowing how basic growth processes respond to increased carbon inputs may also provide a mechanistic basis for the differential phenotypic plasticity exhibited by different plant species/functional types to elevated CO_2.

Keywords: anatomy, development, elevated carbon dioxide, morphogenesis, morphology, ultrastructure

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Introduction

Carbon dioxide, released by anthropogenic combustion processes, has increased from 280 μmol mol^{-1} before the onset of the Industrial Revolution to around 365 μmol mol^{-1} today, and continues to rise at about 1.8 μmol mol^{-1} y^{-1} (Mendelsohn & Rosenberg 1994). Plants exposed to elevated CO_2 often show increased growth and water-use efficiency (Rogers & Dahlman 1993; Allen & Amthor 1995; Wittwer 1995), and increased rates of photosynthesis (Long & Drake 1992; Amthor 1995). Although recent reviews have summarised the effects of elevated CO_2 on ecosystems (Bazzaz 1990; Körner *et al.* 1996), plant herbivore interactions (Bezemer & Jones 1998), crop species (Rogers & Dahlman 1993; Goudriaan & Zadoks 1995), plant biomass accumulation (Poorter 1993), root:shoot ratios (Rogers *et al.* 1997a), and metabolism (Bowes 1991; Farrar & Williams 1991; Long 1991; Stitt 1991; Gunderson & Wullschleger 1994; Amthor 1995), effects of elevated atmospheric CO_2 on plant structure including ultrastructure, anatomy, morphology, and architecture are far less studied. Changes in plant metabolism resulting from increased C availability will inevitably drive changes in plant structure at multiple hierarchical levels contributing to altered plant, community, and ecosystem level function.

Plant morphogenesis is governed by the effects of environmental conditions superimposed upon genetic constraints. Thus, genetically identical plants can exhibit very different structural features when subjected to different environmental conditions. Although genetically determined design constraints define borders of plasticity, most plant species do mediate the effects of environmental conditions impinging upon them by adjusting developmental processes and resultant structural characteristics to some extent. Ability to adjust both metabolically and structurally to resist stressful environments and to exploit abundant resources will dictate the fate of individual plant species as the global environment continues to change.

Plants exposed to elevated atmospheric CO_2 are almost always larger than those grown in ambient CO_2; the magnitude of growth stimulation is typically dependent upon photosynthetic pathway, sink strength, phenotypic plasticity, and plant life history strategies (Hunt *et al.* 1991). Poorter (1993) surveyed the literature (156 plant species) and found the average stimulation of vegetative whole plant growth to be 37%. In addition to increased whole plant biomass, altered root:shoot ratios are often noted, suggesting a shift in the functional relationship between these organs. Rogers *et al.* (1997a) recently reviewed the available literature for crop species and found that root:shoot ratios usually increased (59.5%), sometimes decreased (37.5%), but rarely remained unchanged (3.0%). Although examining plant growth and allocation patterns by assessing total biomass and root:shoot ratios may be a valuable starting point in determining plant response to elevated CO_2, it may be a rather insensitive indicator of what is actually happening to plant growth in terms of structure and function (Stulen & den Hertog 1993; Taylor *et al.* 1994; Sattler & Rutishauser 1997). For example, differences in root architecture including root depth, branching, and morphology may impact patterns of water and nutrient uptake independent of total biomass (Tremmel & Bazzaz 1993).

The ability of plants to respond to future elevated CO_2 levels will undoubtably hinge upon physiological characteristics such as sink strength, efficiency of N and water use, and photosynthetic pathway and capacity, but will, with at least equal importance, depend upon plant structural adaptation (Díaz 1995). Körner (1991) has aptly pointed out that the exclusive use of gas exchange data to predict plant success has been over-valued and over-represented in literature addressing plant response to elevated CO_2. Plant structural responses to elevated CO_2 may prove to be more important than physiological characteristics in natural environments where plants must compete for scarce resources (Díaz 1995; Teugels *et al.* 1995). For example, Reekie & Bazzaz (1989) reported that although growth in elevated CO_2 did not affect photosynthesis or total biomass accumulation in five trees grown individually, when grown in competition with one another, elevated CO_2 induced changes in species composition resulting from changes in canopy structure. Similarly, Küppers (1985) found that success of species in competition was not related to photosynthetic capacity but instead was related to structural characteristics including branching angles, bud activity, leaf positioning, and internode lengths. Plants must compete for common, usually finite, resources in both natural and managed ecosystems, and these resources are acquired through roots (water and nutrients) and shoots (light, CO_2). Thus, it follows that in competition, the ability of plants to increase number, size, and efficiency of modules through which resources are acquired, relative to adjacent plants, will define their competitive ability, and resultant success (Teugels *et al.* 1995).

In addition to effects of elevated CO_2 on structurally mediated competitive relationships, alterations in plant form will feed back on physiological processes at the whole plant level which in turn will dictate further growth, development, and survival of the plant. So, as is often expressed by biologists, form and function are inextricably interwoven; function gives form and form results in function. Consideration of physiological alterations, structural modifications, and their interactions resulting from growth in elevated CO_2 will provide a more holistic concept of how plants will change in response to increasing CO_2, and will help bridge data collected at the physiological level to whole plant and canopy level processes (Murthy & Dougherty 1997).

Plant anatomical, morphological, and architectural adaptations to rising global CO_2 levels may prove to be critical due to the importance of plant form in the acquisition of resources, as a determinate of plant competitive interactions, and as a modifier of metabolic processes. Therefore, the purpose of this manuscript is to review extant data on effects of elevated CO_2 on vegetative plant structure and development. Alterations in plant structural development including ultrastructural, anatomical, morphological, architectural, and canopy levels resulting from exposure to elevated CO_2 will be reviewed and suggestions for further research offered.

Control of whole plant growth: root shoot signalling

It has long been recognized that plant carbon and N availability are crucial to, and perhaps largely control, whole plant growth patterns. A conceptual understanding of how dynamics of plant C and N pools control growth, via differential allocation of resources to shoots and roots, is central to understanding the significance of rising atmospheric CO_2 for plant structure (Fig. 1). It has long been understood by plant biologists that root-to-shoot ratios of plants as well as whole-plant growth rates, both important determinates of plant success, are determined by the relative availabilities of C and N. In general, plants are able to alleviate disequilibrium between growth processes and resource availability by differentially allocating resources to that plant structure through which the most limiting resource is acquired. For example, plants grown in nutrient-limiting soils allocate resources preferentially below-ground in order to pre-empt critical deficiencies. On the other hand, plants growing in fertile soil are functionally C limited and thus allocate energy into leaf growth to acquire C, the most limiting resource for growth. This concept is reinforced by an extensive literature base (Chapin *et al.* 1987; Aiken & Smucker 1996).

Obtaining a meaningful and satisfying understanding of mechanisms underlying plant structural responses to elevated CO_2 requires a conceptual knowledge of the mechanisms that link recognition (increased photosynthesis $\rightarrow$ increased carbon $\rightarrow$ diluted tissue N) with transduction (assimilate partitioning, root-to-shoot signalling, differential gene expression, hormones) with reaction (greater rates of cell division/cell expansion) with adaptation (morphological, anatomical, and ultrastructural). Figure 1 represents a conceptual model illustrating the channels through which increased C input may effect structural change. In some cases, growth responses may be direct (based simply on substrate availability) and significantly more simple than indirect growth responses (based on chemical messengers). Although the complexity of such a holistic view of the interaction of environment with plant growth processes can not be overstated, and perhaps will not be practical for several years, such an approach is vital if form and function are to be linked. In this review, we attempt to understand how plant structure is altered by CO_2-enriched environments by integrating what is known at scales ranging from molecular to canopy. Closing the gap between individual developmental processes (e.g. cell division, cell expansion, and cell differentiation) and whole plant growth patterns is necessary before we can hope to understand the ways in which plants (both in isolation and within competitive arrays) will respond to future elevated CO_2 levels. As stated recently by Körner *et al.* (1996) concerning the state of knowledge about the implications of rising global CO_2 for plants and ecosystems: '…the field is at the portal of an era where further progress in understanding

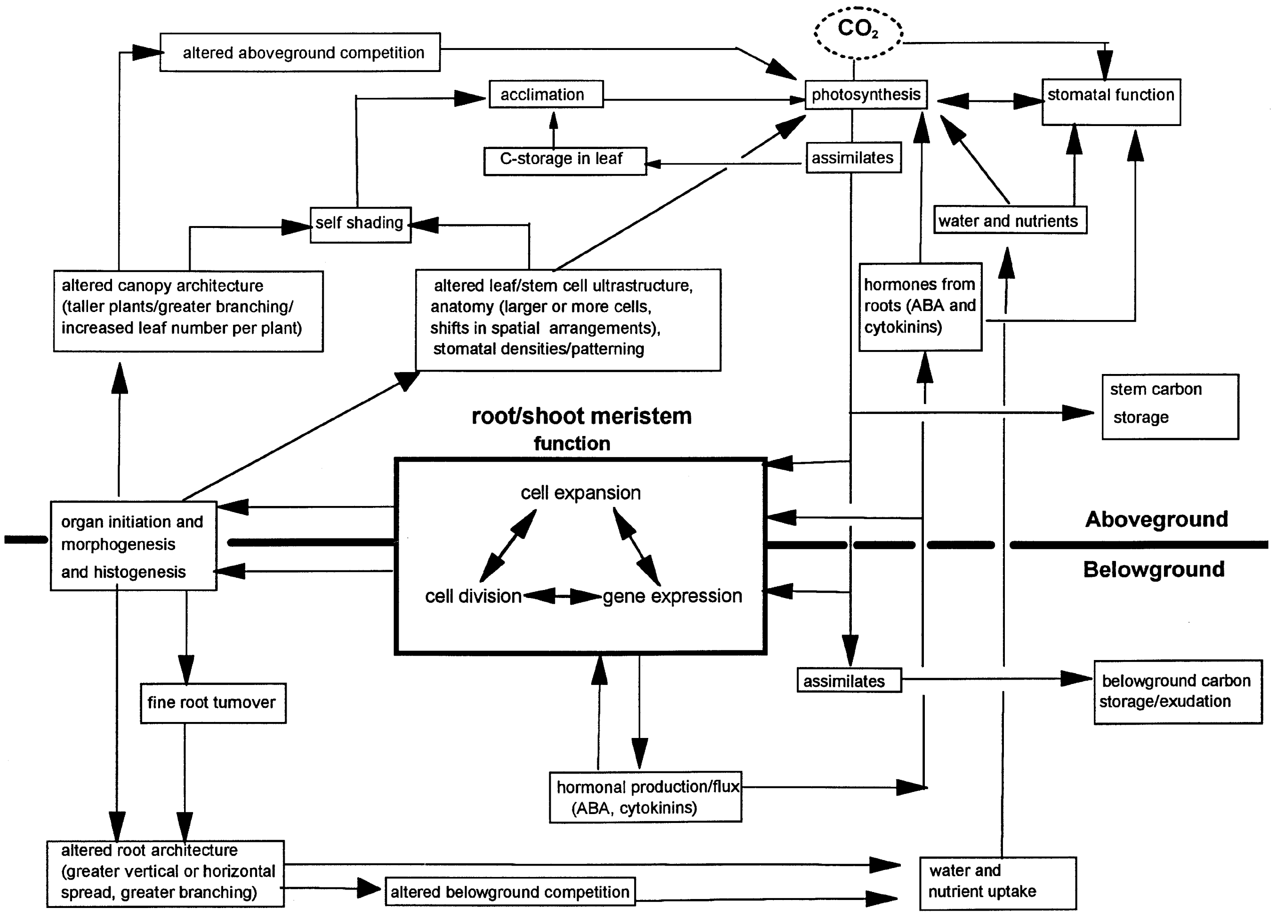

Fig. 1 Conceptual model indicating the channels through which increased atmospheric carbon dioxide availability may effect plant structure. Note the central role of root/shoot meristem function.

CO_2 responses is critically dependent on an effective integration across fields and approaches…'.

Shoot development

Cell division, expansion, and meristem function

Primary stem growth is ultimately the consequence of apical meristem function. Plant development, initiated at meristems, consists of processes that include cell division, controlled cell expansion, and differentiation (Taylor 1997). These interdependent processes are regulated by specific, genetically programmed, timed ontogenetic events (Körner 1991) integrated with environmental cues to affect the rate, shape and number of organs formed by plants (Kerstetter & Hake 1997). It follows that altered plant structure induced by exposure to elevated CO_2 may be the result of greater rates of cell division, increased cell expansion, altered patterns of primordium initiation, altered morphogenesis and histogenesis, or a combination of these processes. There has been some disagreement in the literature about which is impacted by elevated levels of CO_2, thus a brief discussion about factors affecting these processes, in the context of CO_2-induced changes, is necessary.

First of all, **what stimulates cells to divide**? As recently discussed by Jacobs (1997), there are thought to be two nonmutually exclusive answers to this question. First, cell size homeostasis dictates that as cells expand, a size threshold will be reached beyond which cell volumes and surface areas will exceed the capacity of the nucleus to govern cell function. At this threshold, cells would duplicate their organelles and partition the growing space with new cell walls. Thus, expansion of cells may drive mitosis.

Cells, however, may also divide independently of cell expansion cues. It has long been known that plant hormones including cytokinins, auxins, and gibberellins are involved in controlling developmental events within apical meristems such as cell division, cell elongation and protein synthesis. For example, both auxins and cytokinins have been shown to increase expression of cyclin genes (Renaudin *et al.* 1994; Kouchi *et al.* 1995; Kende & Zeevaart 1997). It has recently been established in plants that cyclins, a class of regulatory subunits of a family of protein kinases, facilitate the transition of cells from G0 to G1 of the cell cycle, thus stimulating division (Soni *et al*. 1995; Jacobs 1997). It has been reported that sucrose may also be a chemical control point in the cell division cycle (Francis 1992; Ranasinghe & Taylor 1996), perhaps acting by mediating cyclin activity (Kinsman *et al.* 1997). So, cell division may be stimulated by expansion of cells beyond a threshold point, or by increased cyclin activity alone (Jacobs 1997; Kinsman *et al.* 1997). Based on this information, it is possible that growth stimulation of plants grown in elevated CO_2 may be direct (based on substrate supply) or indirect (based on chemical signals) or both. Considering the large impact that growth in elevated CO_2 has on plant root systems (Rogers *et al.* 1997b), it is possible that root cytokinin production, and flux to shoots, may be altered thereby modifying above-ground growth (Fig. 1).

Although it is thought by many researchers that cell production rate largely dictates growth (Körner 1991; Jacobs 1997; Kinsman *et al.* 1997), to a lesser extent increases in **cell expansion** resulting in larger cell size may also contribute to increased plant and organ size without a concomitant increase in cell production (Ranasinghe & Taylor 1996). Cell expansion is controlled by cell wall loosening, wall extensibility, and the rate at which cells can take up water and solutes (Cosgrove 1993, 1997; Ferris & Taylor 1994; Taylor *et al.* 1994). Cell size and rate of cell expansion are influenced by environmental factors including light, water, and nutrient availability and also by endogenous factors such as hormones. It is difficult to disentangle responses of cell production and cell expansion to elevated CO_2 because these processes are so interdependent (Fig. 1). Depending upon context, cell extension may be driven by mitosis, or mitosis may be driven by cell extension (Jacobs 1997) (Fig. 1). This difficulty has been identified and discussed (Ranasinghe & Taylor 1996). Although many studies have reported stimulated growth of stems and branches in plants grown in elevated CO_2 (Downton, Grant & Chacko 1990; Pushnik *et al.* 1995; Slafer & Rawson 1997), few studies have discerned relative contributions of more cell division from larger cell size. St. Omer & Horvath (1984)) examined cell size of primary stem tissue for *Layia platyglosa* grown in elevated CO_2. No increase in diameter of either xylem or sieve elements was evident. Although cell size was unchanged, cortex width and stele diameter of plants grown in 700 $\mu mol\,mol^{-1}$ CO_2 were 45 and 41% higher than in plants grown in atmospheres containing 300 $\mu mol\,mol^{-1}$ CO_2 implying that cell division alone was enhanced.

Kinsman *et al.* (1997) recently provided evidence indicating that exposure to elevated CO_2 stimulates primary growth of shoots by increasing the proportion of rapidly dividing cells and shortening cell cycle durations in shoot apices. Populations of *Dactylus glomerata* from Portugal exhibited a 1.5–3.0 fold increase in the proportion of rapidly cycling cells in the apical dome while a population from Sweden showed only a 1.2 fold increase when exposed to elevated CO_2. Furthermore, they noted that the cell cycle shortened ≈ 26% in both populations. In the Portuguese population, decreases in the length of the cell cycle resulted almost exclusively from a shortening of the G1 phase, whereas in the Swedish population, both the G1 and G2 phases were shortened. Although the authors did not assay for altered cyclin activity or cytokinin levels or measure tissue carbohydrate levels, they speculated that increased photosynthate availability (i.e. sucrose) in meristems may have increased the proportion of rapidly dividing cells by stimulating cyclin activity. Interestingly, differential increases in total biomass due to exposure to elevated CO_2 paralleled the differential increases in the proportion of dividing cells for the two populations (33 and 21% increases in total biomass for the Portuguese and Swedish populations, respectively). These results suggest that events at the cellular level may provide a key to understanding how growth is controlled at the whole plant level and thereby contribute to a mechanistic understanding of differential phenotypic plasticity exhibited by different species grown at elevated CO_2 concentrations (Körner 1991; Taylor *et al.* 1994). Unfortunately, these results also underscore the problems associated with extending findings observed for one species or population to other related species or populations (Kinsman *et al.* 1996).

Branching

Although it is clear that exposure of plants to elevated CO_2 stimulates cell division at the shoot apical meristem either directly or indirectly, it is not clear how these cells are partitioned at the shoot axis. Undifferentiated cells produced at the shoot apex must undergo transition to a more specialized state in which they either become components of organ primordia or contribute to internodes between organs (Clark 1997). Examining the effects of CO_2 enrichment on internode length relative to lateral branch or leaf initiation may provide clues for understanding cell partitioning at the shoot apices.

Numerous studies have shown increased **stem or branch elongation** in plants grown in elevated CO_2

Table 1 Effects of growth in elevated CO_2 on stem and branch characteristics of woody species

Species	§Location/ Duration	Ambient $[CO_2]$	Elevated $[CO_2]$	Branch/stem diameter	Branch length	Branch number	Plant height	Branching patterns	Anatomical alterations	Reference
Pinus radiata	GC (120 d)	320	640	–	–	–	–	–	trach. length NS trach. diameter NS wood density NS	Donaldson *et al.* (1987)
	GC (22 wks)	330	660	–	–	–	NS	–	wood density +	Conroy *et al.* (1986)
	GH (2 y)	340	660	+	–	+	(–)	whorl # (–) branches/whorl + apical dom. (–)	trach. length NS trach. wall thickness +44% wood density +	Conroy *et al.* (1990a)
Pinus taeda	Phy (1 season)	350	500	+	–	NS	+	–	–	Sionet *et al.* (1985)
			650	+	–	NS	+	–	–	
	Phy (113 d)	350	675	NS	–	–	NS	–	–	Tolley and Strain (1984)
			1000	NS	–	–	NS	–	–	
	gBC (21 m)	360	535	–	–16%*	–	–	flush # NS	bark density NS	Murthy and Dougherty (1997)
			710	–	+15%	–	–	flush # NS	bark density NS	
	Phy (172 d)	375	710	LN: +12%	+47%	1°: +45% 2°: +66%	+15%	plants taller with more and longer 1st and 2nd order branches	–	Larigauderie *et al.* (1994)
				HN: +27%	+53%	1°: 1100% 2°: +56%	+17%			
Pinus ponderosa	GC (6 m)	350	525	NS	–	–	+20%	–	–	Pushnik *et al.* (1995)
Picea glauca	GC (100 d)	350	750	–	–	NS	NS	–	–	Brown and Higginbotham (1986)
Picea rubens	GH (5 m)	362	711	+33%	–	+33%	+23%	buds yielded greater new fixed growth	–	Samuelson and Seiler (1993)
Garcinia mangostana	GC (1 y)	395	800	+26%	+56%	+	+33%	1° node # NA lateral node # +40%	–	Downton *et al.* (1990)
Populus tremuloides	GC (100 d)	350	750	–	–	NS	NS	–	–	Brown and Higginbotham (1986)
Populus trichocarpa	OTC (92 d)	350	700	–	+56%	+	+	–	–	Radoglou and Jarvis (1990)

Species	[§]Location/ Duration	Ambient [CO_2]	Elevated [CO_2]	Branch/stem diameter	Branch length	Branch number	Plant height	Branching patterns	Anatomical alterations	Reference
Ochroma lagopus	GC (60 d)	350	675	–	–	–	NS	–	–	Oberbauer *et al.* (1985)
Pentaclethra macroloba	GC (123 d)	350	675	–	–	–	NS	–	–	
Liquidambar styraciflua	Phy (1 season)	350	500	+	–	NS	+	NS	–	Sionit *et al.* (1985)
			650	+	–	+122%	+	crown shape altered	–	
	Phy (113 d)	350	675	+16%	–	–	+29%	–	–	Tolley and Strain (1984)
			1000	+12%	–	–	+20%	–	–	
Rhizophora mangle	GH (13 m)	350	700	–	NS	+60%	NS	branching angles/symmetry NS stem volume +100% URL+	–	Farnesworth *et al.* (1996)
Castanea sativa	GH (1 season)	350	700	LF: NS	NS	–	NS	NS	–	El Kohen *et al.* (1992)
				HF: +18%	NS	–	NS	NS	–	
	GC (7 m)	350	700	–	–21%	–	–	early cessation of stem growth	–	Mousseau and Enoch (1989)
Betula pendula	GC (79 d)	350	700	–	–	NS	NS	side shoot #: NS canopy shape NS	–	Pettersson and McDonald (1992)
Quercus robur	GH (19 m)	350	700	+175%	+137%	–	+296%	–	increased stem growth rate	Atkinson *et al.* (1997)
	GH (10 m)	350	700	+	–	–	–	–	vessels/stem + vessel lumen area/vessel + area/stem x-sect. +140%	Atkinson and Taylor (1996)
Prunus avium	GH (2 m)	350	700	–	–	–	–	–	vessels/stem NS vessel lumen aea/vessel NS area/stem x-sect. NS	

[§]Plants from all experiments cited in this table were grown in containers except those indicated with a [g] (plants grown in the ground). d, days; m, months; y, years; GC, growth chamber; GH, glass house; OTC, open top chamber; phy, phytotron; BC, branch chamber.
NS, not significant; LN, low nitrogen; HN, high nitrogen; LF, low fertility; HF, high fertility; *, mean of two or more fertility or water treatments.
Trach, tracheid

Table 2 Effects of growth in elevated CO_2 on stem and branch characteristics of nonwoody species

Crop Species	§Location/ Duration	Ambient [CO_2]	Elevated [CO_2]	Branch/stem diameter	Branch length	Branch number	Plant height	Branching patterns	Anatomical alterations	Reference
Glycine max	Phy (18 d)	350	700	+18%	–	–	+15%	node # NS	–	Rogers *et al.* (1992)
Triticum aestivum	GH (1 season)	360	720	–	–	–	+17%	node # NS internode length +	–	Slafer and Rawson (1995)
	OTC (1 season)	360	720	+	–	–	–	tiller #+	–	Kendall *et al.* (1985)
Lolium perenne	GC (3 m)	367	620	–	+	–	–	–	–	Nijs *et al.* (1988)
	GH (1 season)	360	700	–	–	–	NS	NS	–	Teughels *et al.* (1995)
Natural Species										
Agrostis capilaris	GC (23 weeks)	360	610	–	–	–	NS tiller #+	leaf #/tiller +	–	Newberry and Wolfenden (1996)
Layia platyglossa	GC (1 season)	300	700	+22%	–	–	–	–	cortex width +41% stele diameter +41% xylem cell diam. NS sieve element diam. NS phloem width NS	St. Omer and Horvath (1984)
			1400	+66%	–	–	–	–	cortex width +73% stele diameter +111% xylem cell diam. NS sieve element diam. NS phloem width −44%	
Lonicera japonica	GC (54 d)	350	675	–	+300%	+200%	NS	canopy density + branch initiation rate +	–	Sasek and Strain (1991)
			1000	–	+500%	+200%	NS	canopy density + main stem nodes (–)	–	
Lonicera sempervirens	GC (54 d)	350	675	–	+	+300%	+	total branch length + length per branch NS main stem nodes +	–	Sasek and Strain (1991)
			1000	–	+	+300%	+	total branch length + length per branch NS main stem nodes +	–	
Festuca arundinaceae	GH (1 season)	360	700	–	–	–	–	NS	NS	Teugels *et al.* (1995)

§Plants from all experiments cited in this table were grown in containers. d, days; m, months; y, years.; GC, growth chamber; GH, glass house; OTC, open top chamber; phy, phytotron; BC, branch chamber; NS, not significant; LN, low nitrogen; HN, high nitrogen; LF, low fertility; HF, high fertility; *, mean of two or more fertility or water treatments.

without concomitant increases in **node number** (Table 1). For example, Downton *et al.* (1990) reported that *Garcinia mangostana* was 33% taller at elevated CO_2 but that the number of primary nodes was not affected. Rogers *et al.* (1992) reported a 15% increase in plant height for *Glycine max* grown in elevated CO_2 although the number of nodes was unchanged. Similarly, Slafer & Rawson (1997) reported that height of *Triticum aestivum* plants grown in elevated CO_2 increased by 17% resulting from increased internode length, not from greater numbers of nodes. *Lonicera japonica* grown at high CO_2 levels had fewer main stem nodes while plant height was unchanged (Sasek & Strain 1989). Ackerly *et al.* (1992) concluded that increased branch number in *Amaranthus retroflexus* grown in elevated CO_2 was due to effects on overall rate of development, not to changes in pattern of branch initiation. Finally, Sionit *et al.* (1985) observed greater height in *Pinus taeda* grown in elevated CO_2, but no change in total number of branches. These results suggest that the growth and development of cells and tissues below the site of lateral organ formation are stimulated to a greater extent than is the formation of organ primordia at the shoot tip.

Differential stimulation of cell division in different regions of apical meristems may account for altered patterns of **branch initiation** relative to **internode elongation** (discussed above). Kinsman *et al.* (1996) observed that cell doubling times (cdt) for pith rib meristem cells in *Dactylis glomerata* decreased 4.8 fold at 10 C, 6.1 fold at 20 C, and 2 fold at 30 C in plants grown at 700 μmol mol^{-1} CO_2 compared to those grown under ambient CO_2 levels. Cell doubling times in the peripheral meristem zone and central zone (most distal region of apical dome) were not as sensitive to increases in elevated CO_2. The authors suggested that the pith rib meristem zone would be the first to receive the extra photosynthates produced as a result of the increase in atmopheric CO_2, but concluded that further investigation is warranted. The central zone is the most distal region of the apical dome and, ultimately, is the source of all cells below it. The peripheral meristem is the location of organ initiation and overall cell patterning, and the pith meristem is the site of cell division and expansion and contributes to axial and radial primary growth of internodes (Esau 1977). Thus, a decrease in the ratio of cdt in the pith rib meristem to cdt in the central zone and the peripheral zone may partly explain why the ratio of internode length to node (organ) number often increases in plants grown in elevated CO_2. The extent of internodal elongation is perhaps of primary importance in establishing the gross morphology of a species (Esau 1977).

Although node number appears rather insensitive to elevated atmospheric CO_2, several studies have reported that branch initiation and number have been stimulated, while plant height or branch length has decreased or remained unchanged (Tables 1 and 2). *Rhizophora mangle* grown in elevated CO_2 had 60% more branches than ambient grown plants, but plant height and branch length were unaffected (Farnesworth *et al.* 1996). They also reported decreased branch plastochron. In *Agrostis capillaris*, growth in elevated CO_2 increased tiller number by 20% while plant height remained unchanged (Newbery & Wolfenden 1996). Similarly, Conroy *et al.* (1990a) reported higher branch numbers, resulting from more branches per whorl, in *Pinus radiata* grown at high CO_2 even though plant height decreased. Several studies have suggested that CO_2 enrichment may increase the number (*Trifolium repens*, Ryle & Powell 1992; *Quercus alba*, Norby *et al.* 1986; *Pinus radiata*, Conroy *et al.* 1990a; *Rhizophora mangle,* Farnesworth *et al.* 1996) or size (*Quercus alba*, Norby *et al.* 1986) of buds/node. Other studies have reported either no effects of elevated CO_2 on branch number and branch/stem length (*Castanea sativa, Pettersson & McDonald 1992; Picea glauca, Populus tremuloides*, Brown & Higginbotham 1986) or increases in both branch number and branch/stem length (*Populus trichocarpa*, Radoglou & Jarvis 1990a; *Pinus taeda*, Larigauderie *et al.* 1994).

Greater branch elongation and more branches per node resulting from growth in elevated CO_2 may imply **reduced apical dominance** (Tables 1 and 2). Conroy *et al.* (1990a) reported that in *Pinus radiata* grown in elevated CO_2, the apical portion of the main stem was shorter than the branches at the most terminal whorl which they attributed to reduced apical dominance. Anderson (1976) also observed reduced apical dominance of *Pisum sativum* grown in elevated CO_2. Reduced apical dominance in plants grown in CO_2 enriched atmospheres may result from altered hormonal production/transport due to effects on apical meristem function, or from alterations in whole plant carbon allocation. Mousseau & Enoch (1989) suggested that reduced branch length ($\approx$ 21%) of *Castanea sativa* induced by CO_2 enrichment was the result of early cessation of stem growth caused by apical bud necrosis. Axillary bud status (domant or active) and lateral branch elongation are not, however, completely controlled or dominated by auxin produced by and transported from apical meristems; overall plant vigor and cues transduced from root stimulation or an improvement in water status, can stimulate lateral growth activity (Stafstrom 1995).

The above discussion shows that exposure of plants to elevated CO_2 may stimulate elongation of branches and stems without accompanying increases in the number of nodes produced. Figure 2 shows architectural diagrams of morphological data obtained during the late pod fill stage of soybean plants grown at [CO_2] of 349, 645, and 946 ppm under two water regimes, and effectively

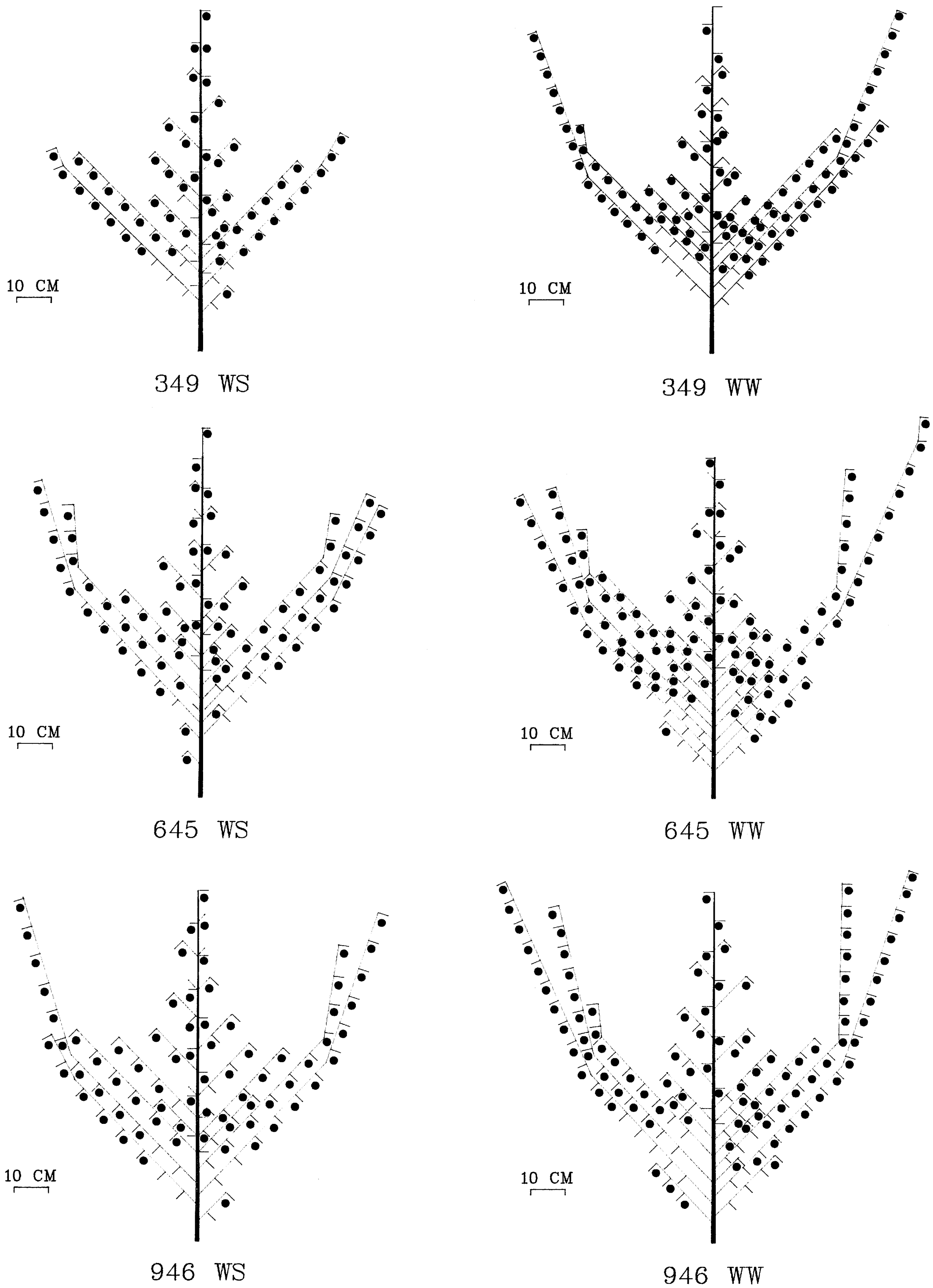

Fig. 2 Diagrams of well-watered (WW) and water-stressed (WS) soybean plants reflecting average morphological data collected at week 14 after planting, later pod fill, from plants grown at 349, 645 and 946 ppm CO_2. Diagonal lines = the sum of the lateral branch lengths at each node; perpendiculars = leaves; dots = 2 pods; scale = 10 cm; $N = 6$ (Prior 1986).

illustrates how branch length is stimulated relative to branch number (Prior 1986). It is important to note however, that although the ratio of height/length to node number appears to increase in plants grown in elevated CO_2, branch number often increases, resulting mainly from greater absolute numbers of nodes per plant, or less frequently, from more buds per node. These alterations, indicating shifts in shoot allometry, have been reported to change canopy shape (*Lonicera japonica*, Sasek & Strain 1991) perhaps contributing to altered competitive relationships between different plant species (*Liquidambar styraciflua* vs. *Pinus taeda*, Sionit *et al.* 1985; Reekie & Bazzaz 1989). Reekie & Bazzaz (1989) grew tropical tree species including *Cecropia obtusifolia*, *Myriocarpa longipes*, *Piper auritum*, *Senna multijuga*, and *Trichospermum mexicanum* in competition at 350, 525, or 700 ppm CO_2. They found that *Senna* decreased in importance resulting from a reduction in the height at which leaves were displayed (Fig. 3). In contrast, *Trichospermum*, *Piper* and *Cecropia* all increased in importance since their leaves were displayed higher (Fig. 3). The authors concluded that shifts in community composition were brought about by alterations in canopy structure due to changes in height growth and branching patterns (Reekie 1996), caused ultimately by alterations in developmental processes within meristematic tissue. Changes in primary stem growth need to be examined and compared for multiple species if we hope to be able to make useful generalizations (based on growth form or functional type) about how CO_2 enrichment affects branching, canopy characteristics, and competitive ability.

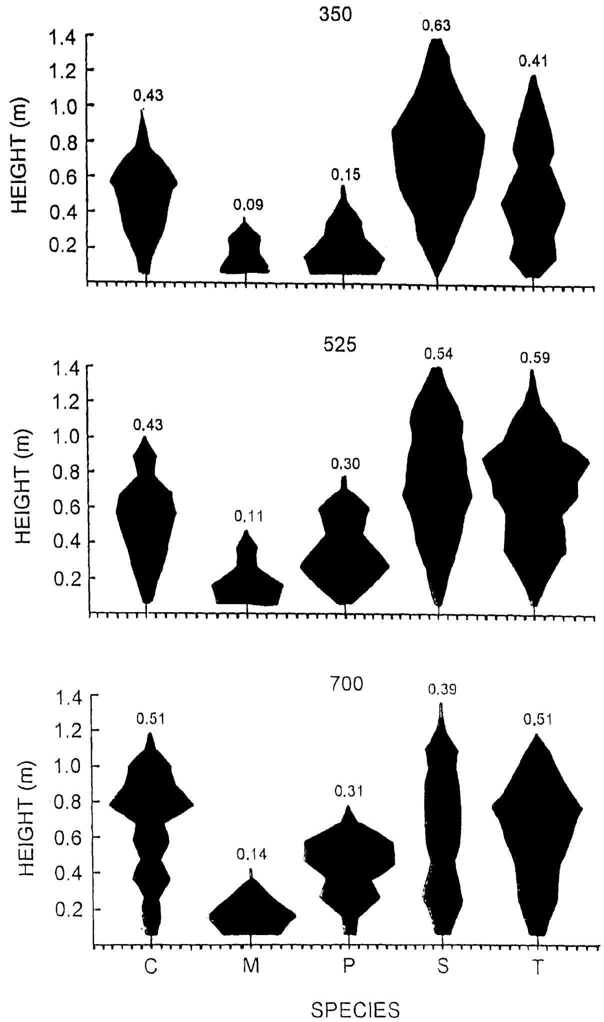

Fig. 3 Effects of atmospheric CO_2 concentration on leaf area profiles of tropical trees. C = *Cecropia*, M = *myriocarpa*, P = *Piper*, S = *Senna*, T = *Trichospermum*. Each tick on the horizontal axis = 0.01 m^2; leaf area was summed over 10 cm intervals. Numbers above each species represent mean canopy height (m). From Reekie & Bazzaz (1989), with permission from Springer-Verlag.

Secondary tissues and anatomy

Below the shoot apex, at the shoot or branch site where elongation (primary growth) has ceased, secondary growth, resulting in the production of secondary xylem (wood) and phloem (bark), occurs by activation of a vascular cambium (Esau 1977). Although most studies concerned with plant structural responses to their environment have focused on leaves, the importance of secondary stem tissue in mediating the flux of resources acquired above and below the ground can not be overstated. In the words of Kozlowski & Pallardy (1997), 'Transport of adequate supplies of metabolites to meristematic tissues where they are used as respiratory subunits and building materials is as important for plant growth as the physiological processes that produce them'. Stem characteristics including anatomical and ultrastructural characteristics of the individual cells which comprise functional domains, in addition to spatial arrangements of the cells and domains that together comprise functional phloem and xylem networks, are crucial. Stems are perhaps the least able to respond to differential atmospheric and edaphic conditions because they grow and are maintained using resources which are all acquired elsewhere; stems must extract required substances through the conduits which pass through them (Cheeseman *et al.* 1996). As such, understanding how stem, leaf and root growth are coordinated represents a complicated problem.

Increases in **diameter of stems and branches** have been reported for many species growing under conditions of elevated CO_2 (Tables 1 and 2) (*Garcinia mangostana*, Downton *et al.* 1990; *Liquidambar styraciflua*, *Pinus taeda*, Sionit *et al.* 1985; *Castanea sativa, Samuelson & Seiler 1993; Quercus robur*, Atkinson *et al.* 1997; *Pinus radiata*,

Table 3 Effects of growth in elevated CO_2 on leaf characteristics of tree species

Species	§Location/ Duration	Ambient [CO_2]	Elevated [CO_2]	Area/leaf	Leaf area/plant	Leaf #/plant	SLA	Other leaf changes	Reference
Pinus ponderosa	GC (6 m)	350	525	–	–33%	–	–14%	mes. area –8%	Pushnik *et al.* (1995)
								vt. area +4%	
	GC (6 m)	"	700	–	–47%	–	–14%		
Pinus taeda	OTC (3 m)	340	520	–	–	–	–	leaf thickness +	Rogers *et al.* (1983)
			718	–	–	–	–	" ++	
			910	–	–	–	–	" +110%	
	Phy (113 d)	350	675	–	NS	–	–9%	Leaf area duration NS	Tolley & Strain (1984)
	Phy (113 d)	″	1000	–	NS	–	–2%		
	Phy (172 d)	375	710	–	LN: +47%	–	–8%	needle dry wt: +58%	Larigauderie *et al.* (1994)
					HN: +66%	–	–8%	" +71%	
	gBC (21 m)	360	535	–	NS	NS	NS	leaf thickness NS	Murthy & Dougherty (1997)
								leaf length NS	
			710	–	+16%	+12%	NS	leaf thickness NS	
								leaf length NS	
	OTC (45 d)	340	520	–	–	–	–	leaf thickness NS	Thomas & Harvey (1983)
								mes. area NS	
								e-tt-vt area NS	
								pid. area (x-sect) NS	
			718	–	–	–	–	leaf thickness NS	
								mes. area NS	
								e-tt-vt area +8%	
								epid. area (x-sect) NS	
			910	–	–	–	–	leaf thickness +10%	
								mes. area +10%	
								e-tt-vt area +11%	
								epid. area (x-sect) NS	
Pinus radiata	GC (22 weeks)	330	660	+15%*	+50%*	+31%*	–16%*	low P: t-tissue –14%	Conroy *et al.* (1986)
								high P: " +23%	
								low P: mes. area NS	
								high P: ʻ +38%	
								low P: vt. area NS	
								high P: ʻNS	
	GH (2 years)	340	660	–	NS	NS	–	–	Conroy *et al.* (1990a)
Pinus palustris	OTC (20 m)	360	720	–	–	–	–	Phloem area –25%	Pritchard *et al.* (1997)
								# sieve cells –26%	
								sieve cell size NS	
	OTC (12 m)	360	720	–	–	–	–	leaf x-sect. area +15%	Pritchard *et al.* (1998)
								fascicle volume +8%	
								t–tis area +3%	
								mes. area +17%¶	
								vt. area +7%¶	
								needle length NS	
	" (20 m)			–	–	–	–	leaf x-sect. area NS	
								fascicle volume –8%	
								t-tis area NS	
								mes. area –17%¶	
								vt. area –10%¶	
								needle length –7%¶	
Picea glauca	GC (100 d)	350	750	–	NA	–	–15%*	leaf wt. ratio NS	Brown and Higginbothem (1986)
Picea rubens	GH (5 m)	362	711	–	–	–	–22%*	–	Samuelson & Seiler (1993)

Species	[§]Location/ Duration	Ambient [CO_2]	Elevated [CO_2]	Area/leaf	Leaf area/plant	Leaf #/plant	SLA	Other leaf changes	Reference
Garcinia mangostana	GC (1 years)	395	800	+10%	+28%	+18%	−19%	–	Downton *et al.* (1990)
Liquidambar styraciflua	OTC (3 m)	340	520	–	–	–	–	leaf thickness +	Rogers *et al.* (1983)
			718	–	–	–	–	" ++	
			910	–	–	–	–	" +121%	
	Phy (113 d)	350	675	NS	NS	+	− 16%	leaf area duration +56%	Tolley & Strain (1984)
			1000	NS	NS	+	− 28%	leaf area duration +22%	
	OTC (45 d)	340	520	–	–	–	–	leaf thickness +17% spongy mes. NS palisade mes. +24% epid. area (x-sect) NS	Thomas & Harvey (1983)
			718	–	–	–	–	leaf thickness +25% spongy mes. +21% palisade mes. +36% epid. area (x-sect) NS	
			910	–	–	–	–	leaf thickness +21% spongy mes. +14% palisade mes. +33% epid. area (x-sect) NS	
Quercus alba	GC (24 weeks)	389	496	–	NS	–	–	–	Norby & O'Neill (1989)
			793	–	NS	–	–	–	
Populus spp.	GC (100 d)	350	750	–	NS	–	−15%*	leaf wt. ratio NS	Brown and Higginbothem (1986)
	gOTC (158 d)	345	693	LN: NS	NS	NS	−12%	leaf area duration NS	Curtis *et al.* (1995)
				HN: +	+35%	++	−16%	leaf area duration +38%	
	OTC (92 d)	350	700	NS	+39%	+11%	(−)	leaf thickness +13% spongy mes. (+) palisade mes. NS epid. area (x-sect) + cell size NS rate of growth +	Radoglou & Jarvis (1990a)
Castanea sativa	GH (1season)	350	700	–	LF: NS	–	NS	–	El Kohen *et al.* (1992)
					HF: +24%	–	NS	–	ʻ
	GC (1 season)	360	700	LF: NS	NS	NS	–	–	El Kohen & Mousseau (1994)
					HF: +24%	+	–	–	
Betula pendula	GC (70 d)	350	700	–	–	NS	−12%	–	Pettersson & McDonald (1992)
Rhizophora mangle	GH (13 m)	350	700	NS	+30%	+15%	NS	mes. area NS epid. area (x-sect) + epid. cell size + #leaves/branch NS leaf area duration NS rate of growth +	Farnesworth *et al.* (1996)
Ochroma lagopus	GC (60 d)	350	675	–	+39%	–	−38%	leaf thickness NS leaf area ratio −22%	Oberbauer *et al.* (1985)
Pentaclethra macroloba	GC (123 d)	350	675	–	NS	–	−20%	leaf thickness NS leaf area ratio −18%	

[§]Plants from all experiments cited in this table were grown in containers unless indicated with a [g] (plants grown in the ground). d, days; m, months; *y*, years.
GC, growth chamber; GH, glass house; OTC, open top chamber; phy, phytotron; BC, branch chamber; NS, not significant; LN, low nitrogen; HN, high nitrogen; LF, low fertility; HF, high fertility; ¶, not statistically significant; *, mean of fertility or water treatments; mes., mesophyll tissue; vt., vascular tissue; e-tt-vt, endodermis-transfussion tissue-vascular tissue; epid, epidermis; t-tis., transfusion tissue; low *P*, low phosphophorus availability; high P, high phosphorous availability. SLA, specific leaf area (leaf area/total leaf dry weight)

Conroy *et al.* 1990a). A few studies have reported that exposure to elevated CO_2 resulted in no effect on secondary growth (i.e. diameter) of stems (*Pinus ponderosa*, Pushnik *et al.* 1995; *Pinus taeda*, Tolley & Strain 1984); and, to our knowledge, no species has exhibited decreased secondary growth in elevated CO_2. Further evidence suggesting an effect of elevated CO_2 on secondary growth of woody stems is provided from tree ring data (reviewed in Weber & Grulke 1995).

There is a paucity of studies on **stem anatomical characteristics** (Tables 1 and 2). Obtaining an understanding of the functional relationship of leaves and stems, and roots and stems, will be impossible until more information on anatomical alterations in stems is available. For example, stem cross-sectional area is thought to be related to whole plant reproductive capacity (Atkinson & Taylor 1996) and is directly proportional to the amount of leaf area which must be supplied with water and solutes (Zimmermann 1983; Atkinson & Taylor 1996). Anatomical characteristics of individual cells within stems are also very important. The ability of stems to transport water to maintain favourable whole plant water relations is governed not only by quantity, but also by the size of xylem conduits (Tyree & Alexander 1993; Atkinson & Taylor 1996).

Tyree & Alexander (1993) hypothesized that increased photosynthesis, increased carbohydrate availability, and resultant increases in the **numbers and sizes of xylem elements** (tracheids and vessel members) within plants grown in elevated CO_2 may enhance the risk of xylem cavitation. Atkinson & Taylor (1996) examined stem characteristics of *Quercus robur* and *Prunus arium* grown under CO_2 enrichment. For *Quercus*, there were greater numbers of vessels in the mid-stem area contributing to a 140% increase in total stem vessel lumen area. Additionally, they found that xylem elements from *Quercus* plants grown in elevated CO_2 had significantly greater mean vessel lumen area than vessels from plants grown with ambient air. These changes contributed to increased hydraulic conductance in plants grown in elevated compared to ambient CO_2. It may also be important to note that increases in total stem vessel area were not related to increases in leaf area. This suggests an uncoupling of the functional relationship of leaves with stems which may imply altered predisposition to xylem cavitation due to drought or freezing stress (Atkinson & Taylor 1996). In contrast, *Prunus* stem total vessel lumen area, vessel number, and lumen area per vessel were not affected by elevated CO_2. Similarly, Donaldson *et al.* (1987) found that CO_2 enrichment caused no differences in tracheid length, tracheid lumen diameter, or tracheid wall thickness in *Pinus radiata*. Conversely, Conroy *et al.* (1990a) reported tracheid wall thickness to increase by 44% in *Pinus radiata* grown under CO_2 enrichment. The authors suggested these inconsistent results were brought about by dissimilar source–sink relationships due to differences in plant age between the two studies. This type of discrepancy is not unusual in the literature on effects of elevated CO_2 on plant processes, both metabolic and structural. Often effects of elevated CO_2 show subtle differences if growth conditions change: pot size (Arp 1991), nutrient and water availability (Prior *et al.* 1997), and different CO_2 exposure systems and concentrations (Ceulemans & Mousseau 1994). Furthermore, whole plant responses to elevated CO_2 may decrease over time due to biochemical (e.g. decreased rubisco activity, Gunderson & Wullschleger 1994), ultrastructural (e.g. chloroplast disruption, Pritchard *et al.* 1997), or canopy level (e.g. self-shading, Newbery & Wolfenden 1996) limitations. There are too few data concerning the effects of CO_2 enrichment on secondary stem anatomy to make generalizations.

In addition to increased stem diameter and altered stem cell sizes, **stem density** may be altered by CO_2 enrichment (Tables 1 and 2). Wood density has been reported to increase (*Liquidambar stryraciflua*, Rogers *et al.* 1983; *Pinus radiata*, Conroy *et al.* 1986) or remain unchanged by growth in elevated CO_2 (*Pinus taeda*, Murthy & Dougherty 1997). In the only known study to examine density of bark, Murthy & Dougherty (1997) reported no change in bark density of *Pinus taeda* grown in elevated CO_2. Density of wood may be an important component of wood quality for both timber and paper production (Conroy *et al.* 1990a,b).

In conclusion, existing data suggest that elevated CO_2 drives increased stem growth primarily by stimulating cell division within shoot apices. However, cell proliferation may be stimulated to different extents throughout different meristematic regions which perhaps accounts for observed increases in nodal elongation relative to branch initiation, and other shifts in whole plant architecture. Furthermore, in some cases, growth in elevated CO_2 appears to stimulate lateral growth suggesting reduced apical dominance. Although it can be inferred that vascular cambium activity is stimulated (increased secondary stem diameters are often observed), there is a profound lack of data concerning the impact of elevated CO_2 on processes driving secondary growth and also on anatomical shifts which may result from altered cambium function.

Leaf development

Why study effects of elevated CO_2 on leaf structure?

Of all plant organs, leaves are the most morphologically diverse (Poethig 1997) and exhibit the greatest structural plasticity in response to disparate environmental conditions (Esau 1977). Leaf development is crucial to plant function since leaves are vital to light interception, photosynthesis, water use, and therefore, total plant productivity (Teskey *et al.* 1987; Murthy & Dougherty 1997). Leaf structural adaptations clearly play a central role in adaptation by plants to changing environments (Lewis 1972; Ticha 1982; Ashton & Berlyn 1994). Functionally, developing leaves are thought to also produce a hormonal signal (i.e. auxin) which stimulates differentiation of xylem. Thus, amount of leaf area dictates stem area produced (Taylor *et al.* 1994; Atkinson & Taylor 1996), assuring functional equilibrium between leaves and stems. In a broader context, rates of leaf development, leaf area duration, and leaf efficiency (a function of anatomy, ultrastructure, and biochemistry) throughout the growing season dictate the rate of canopy closure and the yearly canopy productivity index (CPI, annual production of wood per unit of leaf area). A thorough understanding of how increasing CO_2 will impact the dynamics of leaf initiation, morphogenesis (development of shape), histogenesis (development of internal organization) and phenology will be necessary to: (i) determine the impact of elevated CO_2 on leaf and whole plant function (ii) determine the effects of these functional shifts on ecosystem processes and physiognomy, and (ii) more accurately link vegetation processes with global carbon models and budgets.

Leaf initiation

Leaves usually arise from the surface of meristems as primordial ridges or flattened bumps at predictable intervals (plastochrons) and locations (phyllotaxy) around the axis of the plant (Poethig 1997). Though generally associated with the shoot apical meristem, leaves may arise elsewhere (Clark 1997; Poethig 1997). As recently discussed by Poethig (1997), the relationship between the shoot apical meristem and initiation of leaves has never been established; 'the meristem may represent a region (or type of tissue) in which leaves can spontaneously self-organize rather than a structural entity that makes leaf primordia.' Thus, leaf morphogenesis is discussed separately from shoot morphogenesis in order to reflect this functional discontinuity.

Two models, discussed by Poethig (1997), explain how **leaf primordia** may arise. In the first, it is hypothesized that a field of cells emerges in the meristem having circularly arranged cellulose microfibrils. Such a microfibril arrangement would cause these cells to expand out of the plane of the shoot apical meristem forming the primordial leaf. The second model suggests that leaf primordia may arise as a result of buckling in outer layers of the meristem in response to mechanical stress in the shoot apex caused by an excess of tissue unable to expand laterally. Such biophysical models, in which physical tension across the meristem triggers changes in cell division patterns leading to the initiation of organ primordia, have recently received molecular support (Taylor 1997).

Plants grown in elevated CO_2 typically have increased rates of photosynthesis leading to greater carbohydrate availability (Cave *et al.* 1981). Increases in assimilate transport and carbohydrate availability in shoot apical meristems may increase rates of cell division as discussed above (Kinsman *et al.* 1997). Furthermore, increased tissue water availability resulting from enhanced water-use efficiency (WUE) and increased root proliferation could contribute to greater rates of cell expansion due to increased cell turgor pressure (Sasek & Strain 1989). These factors combined intuitively suggest increased growth, excess tissue, mechanical stress, increased buckling within apical meristems, and therefore increased leaf initiation in plants exposed to elevated CO_2 concentrations. However, as discussed earlier, cell division may be stimulated to a greater extent in pith rib meristems than in the peripheral meristem region (the site of lateral organ initiation).

Although it is difficult to determine from the literature what effect elevated CO_2 has on leaf initiation at apical meristems, it is generally thought that exposure of plants to elevated CO_2 has only small, if any, effects on **rates of leaf initiation** *per se* (Ackerly *et al.* 1992). Studies which provide clues about initiation of leaf primordia relative to stem or branch growth are rare, so it is difficult to determine whether increases in whole plant leaf area result from altered allometric relationships between total stem/branch length and leaf number, or if leaf area simply increases in proportion to greater total branch/stem length. Of 10 reports on leaf area ratio (LAR=total projected leaf area/total plant dry weight), 50% reported decreases, 30% increases, and 20% no effect, resulting in an average net reduction on LAR of 16% (Tables 3, 4, and 5). Summarizing the findings for 20 observations of leaf weight ratio (LWR=leaf weight/total plant weight), decreases were observed for 45% of species, no change for 55% of species, and LWR never increased. Average net decrease in LWR was 10%. Decreases in LAR and LWR suggest that plants allocate less carbon to production of new leaf area at elevated levels of CO_2. So, although faster rates of leaf initiation are sometimes reported, these increases are probably not of the same magnitude as are increases in stem and root growth. Norby (1996) reported the average increase in the canopy productivity index to be 29% in seven tree species grown in elevated CO_2 (650–700 ppm) further suggesting that less leaf area is

Table 4 Effects of growth in elevated CO_2 on leaf characteristics of crop species

Species	[§]Location/ Duration	Ambient $[CO_2]$	Elevated $[CO_2]$	Area/leaf	Leaf area/plant	Leaf #/plant	SLA	Other leaf changes	Reference
Phaseolus vulgaris	GC (25 d)	340	640	+	–	–	–	rate of growth + epid. cell size + XET activity +	Ranisinghe and Taylor (1996)
	GC	400	1200	–	+26%	–	–	–	O'Leary and Knecht (1981)
	OTC (30 d)	350	700	–	LF: +20%	–	–	leaf thickness + songy mes. + palisade mes. + cell # NS cell size + mes. air space (–)	Radoglou and Jarvis (1992)
					HF: +19%	–	–	leaf thickness + spongy mes. + palisade mes + cell # NS cell size + mes. air space (–)	
Lolium perenne	GH (1 season)	360	710	–	+	–	–	leaf inclination NS leaf distribution NS	Teugels *et al.* (1995)
	GC (5 wks)	340	680	NS	–	–	–	leaf length NS leaf width NS epid. cell length NS epid. cell density NS	Ryle and Stanley (1992)
	OTC (35 d) spring leaves	371	700	aT: +80% a+4°C: +14%	–	–	–	mes. area +46%* epid. area +39%*	Ferris *et al.* (1996)
	OTC (35 d) summer leaves	371	700	aT –15% a+4°C: –13%	–	–	–	mes. area –29%* epid. area –14%*	
	Phy (1 season)	340	680	–	–	–	–27%	leaf weight ratio NS	Morison and Gifford (1984a,b)
Festuca arundinacease	GH (1 season)	360	700	–	+	–	–	leaf inclination NS leaf distribution NS	Teugels *et al.* (1995)
Triticum aestivum	GH (1 season)	360	720	–	–	NS	–	leaf development NS spikelets/spike NS	Slafer and Rawson (1997)
	Phy (1 season)	340	680	–	+68%	–	–18%	leaf weight ratio NS	Morison and Gifford (1984a,b)
Trifolium repens	GH (2 m)	350	700	+	+	+	+	–	Jongen *et al.* (1996)
	Phy (1 season)	340	680	–	+39%	–	–14%	leaf weight ratio –14%	Morison and Gifford (1984a,b)
	GC (90 d)	340	680	+5%	+	+30%	–10%	leaves/stolon NS stolons/plant +44%	Ryle and Powell (1992)

Species	[§]Location/ Duration	Ambient $[CO_2]$	Elevated $[CO_2]$	Area/leaf	Leaf area/plant	Leaf #/plant	SLA	Other leaf changes	Reference
Lycopersicon esculentum	GH (29 d)	400	1000	–	–	–	–	leaf thickness + cell size + cell # NS	Madsen (1968)
Gossypium hirsuium	GH (40 d)	330	640	–	+60%	–	–	–	Wong (1979)
	Phy (1 season)	340	680	–	+14%	–	–18%	leaf weight ratio NS	Morison and Gifford (1984a,b)
Zea mays	GH (40 d)	330	640	–	+10%	–	–	–	Wong (1979)
	OTC (3 m)	340	520	–	+	–	–	leaf thickness NS	Rogers *et al*. (1983)
			718	–	++	–	–		
			910	–	+++	–	–		
	OTC (45 d)	340	520	–	–	–	–	leaf thickness NS mes. area –11% epid. area (x-sect) NS	Thomas and Harvey (1983)
			718	–	–	–	–	leaf thickness –9% mes. area –11% epid. area (x-sect) NS	
			910	–	–	–	–	leaf thickness NS mes. area NS epid. area (x-sect) NA	
	Phy (1 season)	340	680	–	+40%	–	NS	leaf weight ratio –13%	Morison and Gifford (1984a,b)
Helianthus annuus	Phy	340	680	–	+14%	–	–18%	leaf weight ratio –17%	Morison and Gifford (1984a,b)
Vigna unguiculata	Phy			–	+56%	–	–22%	leaf weight ratio –14%	
Macroptilium purpureum	Phy			–	+39%	–	NS	leaf weight ratio NS	
Oryza sativa	Phy			–	NS	–	–14%	leaf weight ratio NS	
Phalaris aquatica	Phy			–	+31%	–	NS	leaf weight ratio NS	
Amaranthus spp.	Phy	340	680	–	+15%	–	–11%	leaf weight ratio –15%	
	GH (31 d)	400	700	–	@28°C:+93%	–	–	plastchr. index +19% stem leaf area +43% branch leaf area +180%	Ackerly *et al*. (1992)
					@38°C:–75%	–	–	plastchr. index NS stem leaf area NS branch leaf area –68%	
	GH (1 season)	350	700	–	(–)	–	(–)	leaf area ratio NS	Garbutt *et al*. (1990)
Sorghum bicolor	Phy (1 season)	340	680	–	+29%	–	NS	leaf weight ratio –15%	Morison and Gifford (1984a,b)
Brassica napus	Phy "	"	"	–	+40%	–	–30%	leaf weight ratio NS	
Hordium vulgare	Phy "	"	"	–	+57%	–	–16%	leaf weight ratio NS	
Vicea faba	Phy "	"	"	–	+42%	–	–11%	leaf weight ratio NS	

Table 4. Continued

Species	[§]Location/ Duration	Ambient $[CO_2]$	Elevated $[CO_2]$	Area/leaf	Leaf area/plant	Leaf #/plant	SLA	Other leaf changes	Reference
Pisum sativum	Phy "	"	"	–	+53%	–	–10%	leaf weight ratio NS	
Medicago sativa	Phy "	"	"	–	+75%	–	–44%	leaf weight ratio NS	
Raphinus sativa	Phy "	"	"	–	-	–	–14%	leaf weight ratio –41%	
Glycine max	OTC (45 d)	348	645	–	–	–	–	leaf thickness +	Leadley *et al.* (1987)
								vt area:total leaf area +	
								mes. cell surface area (–)	
	OTC (3 m)	340	520	–	–	–	–	leaf thickness +	Rogers *et al.* (1983)
								palisade mes. area +	
			718	–	–	–	–	leaf thickness ++	
								palisde mes. area +	
			910	–	–	–	–	leaf thickness +131%	
	Phy (18 d)	350	700	+56%	–	–	–	leaf x-sect. area +12%	Rogers *et al.* (1992)
	OTC (45 d)	340	520	–	–	–	–	leaf thickness +35%	Thomas and Harvey (1983)
								palisade mes. area +32%	
								spongy mes. area +45%¶	
								epid. area (x-sect) NS	
			718	–	–	–	–	leaf thickness +33%	
								palisade mes. area +23%	
								spongy mes. area +39%¶	
								epid. area (x-sect) +25%	
			910	–	–	–	–	leaf thickness +31%	
								palisade mes. area +53%	
								spongy mes area NS	
								epid. area (x-sect) NS	
	GC (45 d)	330	800	–	–	–	–37%	leaf thickness +	Vu *et al.* (1989)
								cell # +	
								intercellular space (–)	

[§]Plants from all experiments cited in this table were grown in containers. d, days, m, months, y, years.
SLA, specific leaf area (leaf area/total leaf dry weight); GC, growth chamber; GH, glass house; OTC, open top chamber; phy, phytotron; BC, branch chamber.
NS, not significant; LN, low nitrogen; HN, high nitrogen; LF, low fertility; HF, high fertility; ¶, not statistically significant; *, mean of fertility or water treatments.
aT, ambient temperature; XET, xyloglucan endotransglycosylase.

required to produce a given amount of stem tissue in CO_2 enriched atmospheres.

Leaf expansion

After the primordial leaf is initiated, it grows up into a peg-like leaf axis. This growing leaf axis has an apical meristem at the tip which increases leaf height, and marginal meristems, plate and adaxial meristems which increase leaf width and thickness (Esau 1977). Like all other developing plant organs, growth rates and patterns of leaves are governed by cell division, cell wall loosening, cell wall extensibility, and cell turgor. As mentioned earlier, there has been some discussion concerning which of these processes is most affected by elevated CO_2. This question is perhaps most pertinent for growth of leaves (as opposed to stems and roots) because they exhibit determinate growth. Any deviation in either cell division or cell expansion could cause significant alterations in final size, anatomy, allometric relationships, and resultant leaf function.

Leaf growth is frequently altered by differences in **plant water potential** (Boyer 1968; Yegappan *et al.* 1982; Taylor *et al.* 1994); water limitations lead to inhibition of both cell expansion and cell division (Jones 1985). This, coupled with the observation that growth in elevated CO_2 enhances the efficiency of water use, has led several authors to attribute increased leaf growth for plants grown in elevated CO_2 to greater cell turgor pressure (Madson 1968). For example, Sasek & Strain (1989) reported that turgor pressure in developing leaves of *Pueraria lobata* was twofold greater in plants grown in elevated CO_2 than for those grown in ambient CO_2 which, they suggested, resulted in increased leaf expansion rates and greater leaf expansivity. However, increased turgor pressure can result in increased cell expansion only when accompanied by cell wall relaxation. In fact, biochemical and molecular properties governing cell wall relaxation and expansivity are thought to be of overriding importance in the control of cell growth (Cosgrove 1993, 1997; Taylor *et al.* 1994).

If greater turgor pressure alone is not sufficient to account for the increases in leaf growth commonly reported for plants grown in elevated CO_2, then either **cell wall relaxation, cell division,** or both must be affected. Examining leaf cell size and number may reveal which of these is most affected by elevated CO_2. Increased leaf size associated with larger cells suggests that cell expansion has been stimulated, while increased leaf size associated with more cells may imply stimulation of cell division. Indeed, published studies attribute greater leaf growth to increases in cell number (Tables 3, 4 and 5) (*L. corniculatus*, Taylor *et al.* 1994; *Populus* clones, Ceulemans *et al.* 1995; *Populus*, Gardner *et al.* 1995), and cell size (*Lycopersicum esculentum*, Madson 1968; *Populus* clones, *Radoglou & Jarvis 1992; P. media*, Taylor *et al.* 1994; 3 herbs, Ferris & Taylor 1994) or a combination of both (*Phaseolus vulgaris*, Ranasinghe & Taylor 1996). It appears that no single process has been identified as being responsible for greater leaf lamina size in plants grown in elevated CO_2. Existing studies, however, do suggest that greater cell expansion may play a larger role in effecting larger leaf size that does enhanced cell division (Murray 1997).

Although studies evaluating numbers and sizes of leaf cells in relation to total leaf size may provide insight into the specific growth processes influenced by CO_2 levels, they do not provide a mechanistic basis for this response. Taylor *et al.* (1994) undertook an explanation of the **cellular mechanisms** driving increased cell expansion in leaves. They examined two species exhibiting different responses to elevated CO_2; *Plantago media* leaves increase in size due to greater cell size while leaves of *Anthyllis vulneraria* are unresponsive to elevated CO_2. They found that for *Plantago*, both cell wall plasticity and elasticity were increased in leaves grown in elevated CO_2 implying that cell wall relaxation had occurred. They found no effects of elevated CO_2 on *Anthyllis* cell walls which paralleled its overall lack of response to CO_2 enrichment. Other investigators have temporally separated the processes of cell division and cell expansion in *Phaseolus vulgaris* in an attempt to elucidate the mechanisms underlying increased cell expansion in leaves of plants grown in elevated CO_2 (Taylor *et al.* 1994; Ranasinghe & Taylor 1996). These studies offer three pieces of evidence suggesting that growth in elevated CO_2 caused larger leaf cells by increasing cell wall loosening and extensibility. First, they were able to directly measure increased cell wall extensibility; second, they observed that cell wall yield turgor was reduced; and third, activity of xyloglucan endotransglycosylase (XET) was significantly increased. XET is a putative cell wall loosening enzyme thought to function by cutting and rejoining xyloglucan molecules which connect adjacent microfibrils.

Although data suggest that increases in cell expansion may contribute to larger leaf size more than increased cell division, this is certainly not the case in all species or in the same species at all times of the year (Tables 3,4 and 5). Ferris *et al.* (1996) reported that in *Lolium perenne,* exposure to elevated CO_2 differentially impacted leaf growth in the spring compared to summer. In the spring, leaf area increased due to increased cell expansion, more epidermal cells per leaf, and increased mesophyll area. However, in the summer, there was a negative effect of elevated CO_2 on leaf cell expansion, epidermal cell length, and mesophyll cell area. There is growing evidence for

Table 5 Effects of growth in elevated CO_2 on leaf characteristics of native, non-woody species.

Species	[§]Location/ Duration	Ambient [CO_2]	Elevated [CO_2]	Area/leaf	Leaf area/plant	Leaf #/plant	SLA	Other leaf Changes	Reference
Desmodium paniulatum	Phy (33 d)	350	1000	–	+22%	–	–19%	leaf area ratio –36% leaf area duration +34%	Wulff and Strain (1981)
Lotus corniculatus	GC (3 m)	350	700	–	NS	–	–18%	leaf area ratio –17% leaf weight ratio NS	Carter *et al*, (1997)
	GC (45 d)	345	590	+37%	–	–	–	epid cell # leaf + sd epid cell #/ leaf area +	Ferris and Taylor (1994)
Sanguisorba minor	GC (45 d)	345	590	+31%	–	–	–	epid cell #/ leaf area +	
Anthyllis vulneraria	GC (45 d)	345	590	NS	–	–	–	altered leaf shape epid cell #/ leaf area +	
Lonicera japonica	GC (54 d)	350	675	–	+50%	–	(–)	–	Sasek and Strain (1991)
			1000	–	+50%	–	(–)	–	
Lonicera sempervirensr	GC (54 d)	350	675	–	NS	–	+	–	
			1000	–	NS	–	+	–	
Plantago media	GC (45 d)	345	590	+27%	–	–	–	epid cell #/ leaf area +	Feris and Taylor (1994)
Layia platyglossa	GC (1 season)	300	700	–	–	–	–	leaf thickness +16% vt. area NS phloem area +67% xylem cell diam. NS xylem cell # NS sieve element diam. +29%	St. Omer and Horvath (1984)
Eichhornia cassipes	GC (4 wks)	330	600	NS	+40%	+27%	–	leaf area index +46%	Spencer and Bowes (1986)
Taraxicum officinale	GH (4 m)	350	700	NS	NS	NS	–64%	altered area:perimeter altered length:width leaves ''toothier''	Thomas and Bazzaz (1996)
Pueraria lobata	Phy (45 d)	350	675	+	+22%	–	–	1f. expansion rt. +40% 1f. production rt. +12%	Sasek and Strain (1989)
			1000	+	+54%	–	–	(above values are averaged for the two CO_2 levels)	
Abutilon theophrasti	GH (53 d)	360	700	–	NS	–	NS	vertical distribution of leaf area NS	Hirose *et al*. (1996)

Species	[§]Location/ Duration	Ambient $[CO_2]$	Elevated $[CO_2]$	Area/leaf	Leaf area/plant	Leaf #/plant	SLA	Other leaf Changes	Reference
	GH (1 season)	350	700	–	(–)	–	(–)	leaf area ratio +	Garbutt *et al*. (1990)
	GH (31 d)	400	700	–	18°C: NS 28°C: +55% 38°C: NS	– – –	– – –	plastochron index NS	Ackerly *et al*. (1992)
Ambrosia artemissifolia	GH (53 d)	360	700	–	NS	–	NS	vertical distribution of leaf area NS	Hirose *et al*. (1996)
	GH (1 season)	350	700	–	(–)	–	(–)	leaf area ratio +	Garbutt *et al*. (1990)
Agrostis capillaris	GH (23 wks)	360	610	(–)	NS	+39%*	–	growth rate increased then decreased	Newberry and Wolfenden (1996)
Calamagrostis epigejos	GC (1 m)	350	700	–	–	–	(–)	leaf length + tiller # +36% leaf area ratio (–) relative girth rate +32%	Gloser and Bartàk (1994)
Nymphaea odorata	OTC (5 m)	350	650	+18%	–	+75%[¶]	–	leaf number × lifespan +116%	Idso *et al*. (1990)

[§]Plants from all experiments cited in this table were grown in containers. d, days; m, months; y, years.
SLA, specific leaf area (leaf area/total leaf dry weight); GC, growth chamber; GH, glass house; OTC, open top chamber; phy, phytotron; BC, branch chamber.
NA, not significant; LN, low nitrogen; HN, high nitrogen; LF, low fertility; HF, high fertility; ¶, not statistically significant; *, mean of fertility or water treatments

some species that elevated CO_2 stimulates early expansion of leaves but that the effect then diminishes with time ultimately resulting in leaves of similar size (*Pinus taeda*, Tolley & Strain 1984; *Populus*, Radoglou & Jarvis 1990a; *Populus* clones Taylor *et al.* 1994; *Pinus palustris*, Pritchard *et al.* 1998; *Glycine max*, Sims *et al.* 1998). Nevertheless, increased rates of leaf expansion could significantly impact total plant productivity even if leaves do eventually reach identical fully expanded size (Sasek & Strain 1989).

Lamina size

Regardless of the cellular mechanisms involved, exposure to elevated CO_2 more often than not results in increased **area per leaf**, greater **leaf thickness**, more **leaves per plant**, and higher **total leaf area per plant** (Tables 3, 4 and 5). In 19 reports of surface area per leaf, 58% exhibited greater area per leaf, 37% were not affected, and 11% had decreased leaf area. In two other reports increased area per leaf was reported in fertile soil but was not observed when plants were not fertilized. In 16 reports in which leaf thickness was measured, thickness usually increased (81%), but sometimes was unaffected (19%) by elevated CO_2. For 63 observations of total leaf area per plant, growth in elevated CO_2 resulted in increases more than half the time (57%), sometimes resulted in no change (25%), and 10% decreased; 5% exhibited increases when fertilized and no effect when unfertilized, and 3% of species exhibited increased leaf area at 28 C but decreased area at 38 C. Leaf area increased for one species during the spring, but decreased during the summer. The average net increase in leaf area per plant was 24%. Crop species exhibited the greatest average increase in whole plant leaf area (+37%) compared to trees (+14%) and wild, nonwoody plants (+15%). In addition to altered LAR, LWR, leaf area per plant and leaf size, decreases in specific leaf area (SLA=leaf area/total leaf dry weight) have often been the result of altered anatomy or increased starch accumulation. Of 49 observations, 78% reported a decrease in SLA, 18% showed no significant difference, and 4% increased. This resulted in an average net decrease in SLA (–16%). Tree species and wild, nonwoody species exhibited the most appreciable reduction in SLA (–14% and –20%; Tables 1, 2) compared to crop species (–6%; Table 3). It is generally thought that decreases in specific leaf area result from increased accumulation of leaf total nonstructural carbohydrates (TNC), and accumulation of TNC occurs when C fixation exceeds C utilization. Therefore, crop species would be expected to accumulate less TNC because of rapid growth rates and high sink activity than more slowly growing, nutrient limited natural species.

Leaf ultrastructure

Along with biochemical and biophysical studies, ultrastructural data may provide useful information about cellular processes that drive increased leaf growth at high levels of CO_2. Robertson & Leech (1995) reported increased leaf growth rate in 7-day-old *Triticum aestivum* seedlings which they attributed to increased cell and chloroplast expansion. Although cell and chloroplast profile (cross-sectional) areas were greater, the number, positioning, shape and internal organization (granal stacking) of chloroplasts were unaffected by elevated CO_2. However, chloroplasts from leaves developing in elevated CO_2 contained far less starch than those grown in ambient conditions. An increase in mitochondrial biogenesis in basal leaf cells after only 12 h postmitosis (Robertson *et al.* 1995) suggested that this decrease in starch was probably due to increased rates of respiration. The decrease in chloroplast starch at 7 days resulting from growth in elevated CO_2 was not observed in older (4 weeks) wheat plants. Studies of mature leaves exposed to elevated CO_2 often report more and larger starch grains which in some cases may inhibit **chloroplast structure and function**. For example, several studies have attributed the phenomenon of **photosynthetic acclimation** to elevated CO_2 to disruptions in chloroplast integrity caused by excessive starch accumulation (Cave *et al.* 1981; Wulff & Strain 1981; Ehret & Jolliffe 1985; Yelle *et al.* 1989). Reductions in granal stacking and/or mechanical disruption of chloroplast structure induced by elevated CO_2 may, in some species, only occur when soil N is limiting (Kutík *et al.* 1995), or when both soil N and water are limiting (Pritchard *et al.* 1997). Investigating leaf cell ultrastructure over time, beginning with leaf initiation and ending with leaf sencescence, might prove useful in elucidating the cellular mechanisms involved in leaf expansion, and further, could provide insight into photosynthetic acclimation and the general decrease in response to elevated CO_2 reported to occur over time.

Leaf anatomy

In addition to altered leaf expansion (resulting in changes in leaf size, number, total leaf area per plant, and ultrastructure), changes in internal anatomy and leaf shape are often observed in plants grown in elevated CO_2 (Tables 3, 4, and 5). For example, an extra layer of palisade cells has been observed in *Glycine max* (Rogers, Thomas & Bingham 1983; Thomas & Harvey 1983; Vu *et al.* 1989) and *Castanea sativa* (Mousseau & Enoch 1989) grown in elevated CO_2. Studies typically report increased total **mesophyll cross-sectional area** in leaves from plants grown in elevated CO_2 (*Pinus radiata*, Conroy *et al.* 1986; *Populus trichocarpa*, Radoglou & Jarvis 1990a; *Populus*, Radoglou & Jarvis 1992) although reductions have also been reported (*Pinus ponderosa*, Pushnik *et al.* 1995). However, effects of elevated CO_2 on leaf anatomy may vary depending on stage of leaf development, soil fertility, and season of the year. For instance, Pritchard *et al.* (1998) found that in the early stages of needle development in *Pinus palustris*, elevated CO_2 increased needle fascicle volume by 8% and cross-sectional area by 15% due to increased transfusion tissue area (3%), mesophyll area (17%), and vascular tissue area (7%). But in later stages, fascicle volume was reduced 8% resulting from 7% shorter needles and reduced mesophyll (19%), vascular tissue (10%), and epidermal (19%) cross-sectional areas. Conroy *et al.* (1986) reported that for *Pinus radiata*, mesophyll area was increased by elevated CO_2 when P was nonlimiting, but was not affected when P was limiting. Ferris *et al.* (1996) studied *Lolium perenne*; mesophyll area was increased by high CO_2 concentration in spring, but was decreased in summer.

Vascular tissue area has also been reported to increase in leaves (*Pinus taeda*, Thomas & Harvey 1983; *Pinus radiata*, Conroy *et al.* 1986; *Pinus ponderosa*, Pushnik *et al.* 1995; *Glycine max*, Leadley *et al.* 1987; *Layia platyglosa*, St. Omer & Horvath 1984) and leaf petioles (*Lypersicon esculentum*, Ho 1977). However, Pritchard *et al.* (1997) observed a trend for reduced phloem area in needles of *Pinus palustris* due to fewer, not smaller, cells. They explained these atypical results by suggesting that the production of secondary phloem in the two-year-old needles sampled was negatively impacted by growth in elevated CO_2. The other studies on pine were conducted for shorter durations. Clearly, the lack of data prohibits generalizations about these patterns in either crops or other species.

Increased mesophyll and vascular tissue area commonly reported may be important determinates of both photosynthetic and assimilate transport capacity. However, examining **allometric relationships** between tissue types may be of more use in evaluating the effects of elevated CO_2 on leaf function than studies which simply measure leaf thickness. In one study of these relationships, Leadley *et al.* (1987) reported that although *Glycine max* leaves were thicker when grown in elevated CO_2, they had less palisade cell surface area per unit of leaf area. Internal cell surface area exposed to intercellular spaces is highly correlated with photosynthesis and water use. Additionally, they found that there was a greater ratio of vascular tissue to total leaf cross-sectional area in leaves from plants growing in elevated CO_2. Pushnik *et al.* (1995) also observed an increase in the ratio of vascular tissue cross-sectional area to total needle cross-sectional area in *Pinus ponderosa*. Pritchard *et al.* (1998) reported that for *Pinus palustris*, CO_2 enhancement resulted in a 17% reduction in mesophyll cell surface area per unit of needle volume when N was limiting. No effects, however, were observed on the proportion of needle volume allocated to a given tissue type at any needle age. In other studies in which anatomical allometry was examined, Radoglou & Jarvis (1990a) observed no effect of increased CO_2 level on the ratios of palisade to spongy parenchyma, spongy parenchyma to total leaf thickness, or palisade parenchyma to total leaf thickness in *Populus*.

Stomates

Many studies have reported that growth in elevated CO_2 alters stomatal characteristics. Stomatal density has been observed to increase (Thomas & Harvey 1983; Gaudillére & Mousseau 1989), decrease (Woodward & Bazzaz 1988), or stay the same (Mousseau & Enoch 1989; Radoglou & Jarvis 1990b, 1992; Estiarte *et al.* 1994; Pritchard *et al.* 1998) in plants grown in elevated atmospheric CO_2. Beerling & Woodward (1995) looked at results from historical studies (preindustrial herbarium specimens were compared to contemporary leaves) and experimental studies, and reported that 60% of species show a decline in stomatal density due to elevated CO_2; this number increased to 85% when only experimental data were considered. In addition to altered stomatal frequencies, stomatal patterning may be modified. Boetsch *et al.* (1996) reported that extra subsidiary cells were associated with nearly half of stomatal complexes in *Tradescantia* leaves grown in elevated CO_2 which suggests that recruitment of epidermal cells into stomatal complexes was stimulated. However, Murray (1995) hypothesized that changes in stomatal conductance and WUE resulting from CO_2 enrichment are probably the result of adjustments in stomatal apertures, not consistent morphogenic effects on stomatal abundance. Examination of guard/subsidiary cell ultrastructure, anatomy, and ontogeny with simultaneous observations of gas exchange may provide a more complete understanding of CO_2 effects on biophysical and biochemical aspects of stomatal functioning. Modelling future responses of vegetation will require a sound understanding of how stomatal anatomy and function are affected by higher CO_2 levels (Wagner *et al.* 1996).

Leaf shape

Few studies have examined the effects of elevated CO_2 on leaf shape even though morphological changes in leaf shape may be of greater functional significance than changes in leaf level photosynthesis (Niklas & Owens 1989; Niklas 1989; Thomas & Bazzaz 1996). There is some evidence that carbohydrate availability may be critical in determining leaf form, at least in species exhibiting heteroblastic leaf development (Thomas & Bazzaz 1996). Moreover, Ranasinghe & Taylor (1996) found that spatial patterns of cell wall extensibility were altered in leaves from plants grown in elevated CO_2 which they suggested could lead to alterations in leaf shape. Thomas & Bazzaz (1996) observed shifts in allometric relationships between leaf area and perimeter, and between leaf length and width in *Taraxicum officinale*; and between leaf width and area, leaf length and area, and leaf length and width in *Plantago major* exposed to elevated CO_2 which caused leaves to be more dissected. In contrast, Leadley & Reynolds (1989) found no differences in allometric relationships among length, width, and area of *Glycine max* leaves. It is clear that more attention should be paid to leaf shape in studies examining effects of elevated CO_2 on plant structure and function.

In conclusion, although growth in CO_2-enriched atmospheres results in more leaves per plant, leaf initiation is usually reduced relative to whole plant growth. Although highly variable, rates and magnitude of leaf expansion are enhanced (at least temporarily); this results more often from increased cell expansion than increased cell division. Increased expansion appears to result from greater cell wall relaxation and/or greater cell turgor. Stomatal densities usually decrease, and mesophyll and vascular tissue cross-sectional areas increase contributing to greater total leaf thickness. Although thicker, leaf organization may suggest structural shifts that may limit plant capacity to assimilate carbon (acclimation). Very little is known concerning the impact of elevated CO_2 on leaf shape, or if growth in elevated CO_2 will differentially influence different leaf types (for example, compound vs. simple, opposite vs. alternate).

Root development

Primary growth of roots

Plant roots play a crucial role as the interface between lithosphere and biosphere. Spatial and temporal root structural characteristics govern both plant and soil processes including: (i) root weathering of soil; (ii) input of carbon to soil; (iii) mining soil for resources; and (iv) erosion (Rogers *et al.* 1992). And although roots often exhibit the greatest relative increase in biomass of all plant organs when grown in elevated CO_2, there are few studies on root structural responses, and thus these responses are poorly characterized (Rogers *et al.* 1994; 1997b; Rogers *et al.* 1997a). Reviews have evaluated the effects of elevated CO_2 on roots, and each echoes the need to further investigate below-ground processes, including root structure (Stulen & den Hertog 1993; Rogers *et al.* 1994; Rogers *et al.* 1997b). For a comprehensive tabular summary of CO_2 effects on roots please refer to Rogers *et al.* 1994.

The **root apical meristem** is structurally and functionally different from the shoot apical meristem. In shoots, branching patterns are determined by events at the shoot apical meristem, whereas in roots, lateral organs are initiated in the pericycle, some distance distal to the root tip (Taylor 1997). Functionally, the root axis exhibits negative gravitropism while the shoot axis is positively gravitropic. Finally, roots are thought to play a major role in regulating the growth of above-ground plant organs. Roots sense the availability of soil water and nutrients, and accordingly alter production and transport of hormones such as cytokinins and ABA to shoots, thereby modulating the activity of meristematic tissues above the ground, as well as expression of genes coding for photosynthetic enzymes phosphoenolpyruvate carboxylase, carbonic anhydrase, and the small Rubisco subunit (Aiken & Smucker 1996).

Because of structural and functional differences between the shoot apical meristem and the root apical meristem, one might expect that the effects of elevated CO_2 on components of development leading to increased shoot growth and altered patterns of branching above-ground may not be analogous to below-ground processes. Ferris & Taylor (1994) suggested that cell expansion may be stimulated to a greater extent than cell division in roots. They observed increased root extension rates in *Sanguisorba minor*, *Lotus corniculatus*, *Anthyllis vulneraria,* and *Plantago media* grown in elevated CO_2. They found that cell length was unaffected by CO_2 treatment from ≈ 15–$25\,\mu m$ up to $\approx 1\,mm$ behind the growing tip. Beyond 1 mm, cell length increased at a greater rate in all four species grown in elevated compared to ambient CO_2. Also, they observed increased turgor pressure in plants grown in elevated compared to ambient CO_2. They concluded that stimulation of root growth was the result of increased cell expansion caused by cell wall loosening, in concert with higher cell turgor pressure, rather than by increased cell division. Crookshanks *et al.* (1998) reported a 40% greater mean root cortical cell length in elevated compared to ambient CO_2 at a distance of $375\,\mu m$ from the root tip which they attributed to increased cell wall extensibility but final cortical cell length, however, was unaltered by exposure to elevated CO_2. Kinsman *et al.* (1997) reported that increased root growth in *Dactylis glomerata* was mainly the result of a greater proportion of dividing cells in the apical meristem. Although they also observed greater mitotic indices indicative of shortened cell cycles, these effects were not of sufficient magnitude to cause significant changes in root growth. As shown by the contradictory results of these three studies, further work is needed to elucidate the cellular mechanisms underlying effects of elevated atmospheric CO_2 on stimulation of root elongation behind the apical meristem and also on the events controlling stimulation of lateral root formation in the pericycle. Use of *Arabidopsis thaliana* mutants to elucidate the specific cellular events leading to shifts in root growth as described by Crookshanks *et al.* (1998) currently holds great promise [see also Schiefelbein *et al.* (1997) concerning use of *Arabidopsis* mutants to study mechanistic controls on root development].

Regardless of the cellular mechanisms involved, exposure of the plant canopy to elevated CO_2 usually stimulates growth of roots. Rogers *et al.* (1992) reported a 27% increase in **root diameter** in the root hair zone, a 23% increase in stele diameter, and a 28% increase in cortex width in *Glycine max* grown in elevated CO_2. Increased root diameters have also been reported for *Pinus taeda* (Larigauderie *et al.* 1994). These results notwithstanding, St. Omer & Horvath (1984) found no difference in stele diameter, diameter of tracheary elements, or wall thickness of tracheary elements of *Layia platyglossa* roots grown at higher than ambient CO_2 levels.

Besides greater root diameters, increased **total root lengths** are often observed in plants grown in elevated CO_2 (*Glycine max*, Rogers *et al.* 1992; *Senecio vulgaris*, Bernston & Woodward 1992; *Trifolium repens*, Jongen, Fay & Jones 1996). This may not be true for all species or for roots at all depths within the same species. For example, in *Pinus taeda* grown in elevated CO_2, the upper lateral root fraction increased but the proportion of remaining root components generally declined (Larigauderie *et al.* 1994). Mo *et al.* (1992) reported that, although there was no effect of elevated CO_2 on dry weight in the 0–40 cm depth range, root length in the 0–10 cm range decreased 31% in an assemblage of tallgrass prairie species.

Differential effects of CO_2 concentration on root branching may lead to altered root architecture and altered ability of roots to acquire water and nutrients from the soil profile. For example, Bernston & Woodward (1992) observed for *Senecio vulgaris* that exposure to elevated CO_2 caused more horizontal branching angles of roots contributing to greater horizontal root spread, and Rogers *et al.* (1992) observed longer second-order laterals in *Glycine max*, which could lead to deeper root penetration and thus greater exploration of soil for nutrients and water. Conversely, Del Castillo *et al.* (1989) reported that *Glycine max* grown in elevated CO_2 had more roots due to increased branching rather than longer roots. Increased root numbers may enable plants to more efficiently explore and mine the same volume of soil instead of stimulating exploration into soil deeper or further away.

It is important that more studies focus on effects of elevated CO_2 on root system architecture. The extent of root branching has major implications for the efficiency of water and mineral extraction from soil. Additionally, altered rooting patterns may contribute to root overlap between adjacent plants, possibly intensifying belowground competition (Bernston & Woodward 1992). Perhaps the 31% reduction in root length in the top 10 cm of soil in the assemblage of prairie species resulting from elevated CO_2 (Mo *et al.* 1992) portends altered root competition.

Limitations on carbon assimilation

Limitations imposed by physiology or structure

This review has focused primarily on the ways in which increased plant carbon assimilation may alter developmental processes and ultimately plant structure. Different plant species, however, are not able to assimilate carbon to the same extent. Limitations may result from differing physiological strategies employed to fix carbon (e.g. C3 vs. C4 vs. CAM photosynthesis) (Poorter 1993), or from differential capacity to metabolize carbohydrates by actively growing plant parts. For example, plants which have rapid growth rates or very large storage organs are not as prone to photosynthetic acclimation to elevated CO_2 as slow-growing species with weaker sinks. Photosynthesis, as with most other biochemical processes in living organisms, is subject to end-product inhibition. If plants are unable to utilize fixed carbon for growth, the result is typically an increase in total nonstructural carbohydrates (and perhaps increased C-based secondary compounds) (Poorter *et al.* 1997). Leaf starch accumulation, as discussed earlier, may inhibit photosynthesis by mechanically altering the integrity of chloroplasts, and soluble sugars may inhibit photosynthesis by biochemical feedback processes acting at the level of gene expression. Feedback inhibition of photosynthesis may include adjustments in Rubisco amount or activity, reductions in components of photosystem II (Pennanen *et al.* 1993; Van Oosten *et al.* 1994), reductions in thylakoid stacking within chloroplasts (Wulff & Strain 1981; Kutík *et al.* 1995; Pritchard *et al.* 1997), or a shortage of cytosolic inorganic phosphates (Stitt 1991).

In addition to limitations on carbon assimilation imposed by mechanical and biochemical processes within source leaves and from limitations resulting from weak sink activity, there may be other **structural/**

functional attributes that may also limit plant capacity to exploit the extra carbon available in a higher CO_2 world. For example, Körner *et al.* (1995) found that, following exposure to elevated CO_2, species which load phloem symplastically accumulated 41% total leaf non-structural carbohydrates compared to 25% in species which exhibit apoplastic phloem loading (see also Poorter *et al.* 1997). This implies that plant species may be 'predisposed' to respond to increasing atmospheric CO_2 based not only on photosynthetic capacity and sink strength, but also on the efficiency of assimilate transport from sources to sinks. Pritchard *et al.* (1997) suggested that differences in structure (e.g. vein configurations, plasmodesmatal connectivity, sieve cell organization and form, absence or presence of P-protein), that reflect different strategies for short-distance assimilate transport, phloem loading, and long-distance phloem transport, may, in part, account for observed differences in response patterns of woody broadleaf plants and conifers (Ceulemans & Mousseau 1994; Pushnik *et al.* 1995). Clearly, future studies should attempt to understand plant response to elevated CO_2 not only in the context of plant physiological strategy (photosynthetic pathway) or patterns of biomass accumulation, but also in the context of plant structural attributes.

Limitations arising as experimental artifacts

In almost all of the studies reviewed here, plants were grown individually in pots. This could cause concern for two reasons: (i) first, container grown plants may exhibit greater down-regulation in rates of photosynthesis due to a source-sink imbalance resulting from root constriction (Arp 1991), and/or from nutrient limitations that sometimes plague pot studies (McConnaughay *et al.* 1993), or (ii) individually grown plants may exhibit greater plasticity than plants grown in competition. Furthermore, the tree species studied were typically seedlings and therefore, may not accurately reflect structural responses of mature trees. Moreover, in many cases, plant growth responses to elevated CO_2 may be mediated by nutrient availability (Conroy *et al.* 1990b; Prior *et al.* 1997; Sims *et al.* 1998) which many published studies have not adequately addressed. It is becoming evident that experimental approaches must go beyond simply evaluating the response of isolated container grown plants to elevated CO_2 levels in favour of in-ground studies in which plants are grown at densities reflective of either the natural or agricultural ecosystems where they typically occur. Recently, FACE (free air CO_2 enrichment) studies, and open top field chambers are beginning to be used more regularly to conduct multi-factor in-ground experiments or to study entire ecosystems (see Saxe *et al.* 1998 for a recent discussion on experimental methodology). These studies will fill important gaps in our understanding of plant response to global change, and these new data will likely modify our existing understanding of plant physiological and structural responses to elevated CO_2 levels.

Research recommendations

Review of the extant data on the influence of elevated atmospheric CO_2 levels on plants has revealed the need for focused efforts on nearly every aspect of plant development and structure. We recommend that the following be taken into consideration in future research on the effects of elevated CO_2 on plant structure.

1 How shifts in cellular (cell division and cell expansion) and higher level (morphogenesis and histogenesis) growth processes contribute to alterations in leaf, branch, stem, and root structure.

2 Whether increased cell division is driven by greater rates of cell expansion or is transduced via molecular cues such as sucrose, cyclins, XET, expansins, cytokinins or other molecules important in controlling the cell cycle and cell wall mechanical properties.

3 How cell patterning is altered during morphogenesis and histogenesis resulting in plant organs with different shapes and anatomical organizations, and how cells are partitioned to branch and leaf primordia vs. internodes.

4 Determine if whole plant growth responses to elevated CO_2 may be predictable based on specific developmental events occurring within meristematic tissues. This may prove useful in the search for plant functional types.

5 Alterations in plant allometry including shifts in scaling relationships evident at subcellular (e.g. chloroplasts:mitochondria), anatomical, and morphological levels. Structural alterations in plant organs resulting from growth in elevated CO_2 must be considered in the context of whole plant responses instead of considering single structures or processes in isolation.

6 Anatomical studies of primary and secondary stem and branch anatomy in conjunction with measures of hydraulic conductivity will facilitate a more complete understanding of plant resistance to drought and frost as well as revealing potential shifts in functional relationships of leaves and stems.

7 It has been suggested that compound leaves have a much greater capacity for indeterminate growth than simple leaves (Poethig 1997). Is there a differential CO_2 response between plants with compound vs. simple leaves?

8 The role of ultrastructural, anatomical, and morphological leaf adjustments in the downregulation of photosynthesis rates often observed after prolonged exposure to elevated CO_2.

9 Alterations in plant micromorphology including, but not limited to, root hair and leaf trichome density and structure. Trichomes are vital in protecting plants against pathogens and herbivores and root hair characteristics have a major influence on water and mineral uptake.

Conclusions

It is clear from this review that the effects of elevated CO_2 on plant development and structure are both many and varied. The necessity of understanding the influence of growth in elevated CO_2 on cellular developmental processes, and bridging these basic growth mechanisms to higher level structure and function, is emerging. Ackerly *et al.* (1992) were correct in their assertion that 'elucidation of the relationship between individual developmental processes and whole plant growth has proven much more difficult than the comparable analysis of the mechanistic basis of carbon assimilation and water relations.' However, in spite of these difficulties, several recent studies have provided inroads towards disentangling the effects of elevated CO_2 on the separate but interdependent processes governing growth and development including cell division, cell expansion, primordium initiation, cell differentiation, and organogenesis. The most significant direct effect of elevated CO_2 on plant growth is certainly an increase in carbohydrate availability and increased water-use efficiency. Ultimately, both increased carbon and more efficient water use combine to stimulate cell proliferation either by promoting cell division, cell expansion, or both. Atmospheric CO_2 levels predicted for the next century therefore will likely result in faster seasonal, and successional canopy development and closure. The ability of a given species within the canopy to exploit extra carbon, however, will largely be a function of its inherent physiological and structural attributes integrated with anatomical/morphological plasticity. Some species are likely to overtop others. A more thorough mechanistic understanding of the ways elevated CO_2 will impact structure will emerge as plant biologists develop a better knowledge of how plant developmental processes are regulated.

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